REMARKS

Claims 17, 18, 20, and 85-94 are pending. Claim 17 is amended as discussed below in the discussion of the rejections. Support for the amendment to claim 17 exists in the specification as filed, as discussed below. Claims 89-91, 93 and 94 are amended to conform with the changes made to claim 17. No new matter is added.

Claim 95 is new. Support for this claim exists throughout the specification where it is demonstrated that the multipotent neural stem cells cultured using the claimed method are capable of differentiating into astrocytes (e.g. see Figure 3).

Summary of the Invention

At the outset, Applicants would like to re-summarize the invention, referring to the attached diagram labeled Appendix A. It is believed that this will assist the Examiner in understanding the arguments raised below in response to the rejections and will help illustrate the significance and patentability of the claimed invention. Reference is also made to the Science article which was provided as Appendix A to the Rule 132 declaration of Dr. Reynolds submitted with the Preliminary Amendment to this Rule 62 continuation application (hereinafter referred to as "the Science article").

Fetal, juvenile or adult neural tissue obtained from an animal or human is dissociated and cultured in a growth factor-containing medium. A culture of cells obtained from living tissue is referred to as a "primary culture" by those of ordinary skill in the art. In the presence of a growth factor and suitable culture conditions, multipotent neural stem cells within the primary culture begin to proliferate, while other cells die or remain relatively quiescent (i.e. they may divide only a few times). As indicated in

the first full sentence in column 3 on page 1707 of the Science article, only 15 ± 2 cells out of 1,000 cells from a primary culture of adult mice striata (i.e. approx. 1.5%) proliferate.

Referring to the diagram, a multipotent neural stem cell is depicted as an X within a circle. In the presence of a growth factor, the multipotent neural stem cell proliferates (A) to form a cluster of daughter cells termed a "neurosphere." As indicated by the key of the diagram, the neurosphere contains daughter multipotent neural stem cells and daughter committed progenitor cells (depicted as a dot within a circle).[1] Collectively, the cells of a neurosphere are termed "multipotent neural stem cell progeny", as indeed they are derived from a single multipotent neural stem cell. This proliferation scheme is referred to as "asymmetrical division", a known stem cell characteristic (see Exhibits A-C supplied with the Applicants' Preliminary Amendment, which discuss stem cell characteristics).

Both the specification and the Science article describe the dissociation of neurospheres and resuspension of the cells into fresh growth-factor containing culture medium to form secondary cultures (which comprise suspensions of multipotent neural stem cell progeny). This is discussed in more detail below in response to the rejections raised under § 112, 1st ¶. The passaging of the cells from the primary culture to the

[1] At the time this application was originally filed in 1991, investigators often used the terms "stem cells", "progenitor cells" and "precursor cells" interchangeably to refer generically to undifferentiated cells. However, there are different types of undifferentiated cells. The present application defines the term "progenitor cell" to mean "an oligopotent or multipotent stem cell which is able to divide without limit and under specific conditions can produce daughter cells which terminally differentiate into neurons and glia." (See page 19, lines 12-22). The capability of an undifferentiated cell to divide without limit and produce daughter cells which terminally differentiate into neurons and glia is a stem cell characteristic. This is to be contrasted with a "committed progenitor cell", which, as used in Appendix A and in the present discussion, refers to an undifferentiated cell that may be able to divide to a limited extent, but is committed to a particular differentiative pathway.

secondary culture is depicted in step B of the diagram. It is also described on page 26, lines 21-24 of the specification and on page 1708 of the Science article (the relevant portion has been reproduced below on pages 16 and 17 in response to the § 112 objection to the specification). In the continued presence of a growth factor, the multipotent neural stem cells of the secondary culture further proliferate (C) to form new neurospheres comprised of daughter multipotent neural stem cells and daughter progenitor cells. In contrast, the daughter progenitor cells of the secondary culture are undifferentiated cells that have only a limited ability to proliferate. They may undergo a few divisions (D), but they will not proliferate and form new neurospheres. A progenitor cell is committed to a particular differentiative pathway (E).

The proliferation, dissociation, and reinitiation of proliferation (steps A through C) can be repeated continuously. As stated on page 6 of the Rule 132 declaration of Dr. Reynolds that was submitted with the Preliminary Amendment:

> The cells cultured using the methods described in the application exhibit the characteristics of stem cells: they have the capacity for proliferation, self-maintenance and the production of a large number of differentiated progeny. The culturing and passaging of the neurospheres as described in the specification at Example 5 to line 17 of Example 6 has been repeated over 30 times over the course of 8 months with a[n] arithmetic increase in the total number of viable undifferentiated cells.... While we have not calculated the total number of cells produced from a single multipotent CNS stem cell using these methods, we estimate that after 30 DIV, greater than 0.5 million cells are generated.

Thus, using the claimed methods, large quantities of undifferentiated neural cells can be obtained from just a small amount of neural tissue. This is a significant achievement, particularly if it is necessary to obtain the neural

tissue from a human patient, or in view of ethical considerations, from aborted human fetal tissue.

Referring again to the diagram, when culture conditions are changed to induce differentiation, such as by providing a suitable substrate onto which the cells can adhere, the progenitor cells may further divide to a limited extent (D), but eventually differentiate (E) into neurons or glial cells (i.e. astrocytes and/or oligodendrocytes).

As depicted in the diagram, and described in the specification, using the Applicants' claimed culture method, a single multipotent neural stem cell obtained from dissociated mammalian neural tissue, can be proliferated *in vitro* in the presence of a growth factor to produce progeny (i.e. daughter cells), the progeny can be further proliferated in a secondary culture containing a growth factor. The progeny are capable of ultimately differentiating into neurons and glial cells.

Rejections under 35 U.S.C. § 101

Applicants acknowledge the provisional obviousness-type double patenting rejections raised on page 3 of the office action. Applicants will attend to this rejection in an appropriate manner upon indication of allowable subject matter in this or the related co-pending applications. Applicants draw the Examiner's attention to the following related applications: U.S. Ser. Nos. 08/481,893; 08/486,648; 08/483,122; 08/486,307; 08/484,210; 08/484,406; 08/479,795; 08/480,172; 08/479,796; 08/483,817; 08/484,203; and 08/486,313. These related applications were filed on June 7, 1995. Each application is the same and includes the specification of the present application, as well as the specifications of the copending applications cited on page 3 of the office action.

Rejections under 35 U.S.C. § 112

On page 3 of the present office action, the Examiner maintains the rejection of claims 17, 18, 20 and 85, that were raised in the previous office action (Paper No. 13). Additional rejections under § 112 are raised on pages 10 and 11 of the present office action. All of these rejections are addressed below.

Rejection of Claims 17, 18, 20 and 85-94 under § 112, 2nd ¶:

Prior to addressing the rejections under § 112, 1st ¶, the rejections under the 2nd ¶ (raised on page 11 of the office action) will be addressed, as the claims have been amended and the new language used in the claims will be used in discussing the rejections under the 1st ¶.

The Examiner stated that the claims are indefinite because "the word 'progeny' is vague and unclear since it is not evident if progeny refers to progenitor cells or other stem cells." The Examiner stated further that "the preamble claims a method for the in vitro proliferation of a multipotent stem cell but the body of the claim claims progeny."

Step (d) of Claim 17 is amended to recite that the multipotent neural stem cell is proliferated "to produce progeny which includes daughter multipotent neural stem cells." It is known in the art that when a stem cell divides, "each daughter has a choice: it can either remain a stem cell like its parent, or it can embark on a course leading irreversibly to terminal differentiation." [See page 911 of Exhibit A, (pages from Molecular Biology of the Cell) which was provided with the Preliminary Amendment]. In other words, the progeny of a stem cell includes both daughter stem cells and daughter committed progenitor cells. This is also depicted in the diagram of attached Appendix A. New step (e) of Claim 17 clarifies that it is the "daughter multipotent neural stem cells" that further proliferate. As the term

"daughter cell" is an art-recognized term, and its use in the claims is consistent with the discussion in the specification, use of this term in the claims is proper.

The claims are also rejected under § 112, 2nd ¶ because the Examiner believes that the term "passaging" lacks support in the specification because there is no discussion of trypsinization of the cells. Trypsin is applied to adherent cells to remove them from culture dishes so that they can be transferred to new culture media. In the absence of an adhesive substrate, neurospheres are floating clusters of cells that do not adhere to the tissue culture plates. Therefore, there is no need to trypsinize them in order to passage them. In any event, claim 17 has been amended to use the Examiner's suggested term "transferring". Support for this term is on page 41, lines 19-28, where the preparation of secondary cultures in fresh medium is described.

Claim 17 is also amended to clarify that step (c) involves the preparation of a "primary culture", and step (e) involves the preparation of a "secondary culture". While the terms "primary cultures" and "secondary cultures" are not used in the specification, they are terms that are commonly used in the art. This is evidenced by the attached Appendix B (p. 161), which is a portion of a university-level molecular biology text book, Molecular Biology of the Cell, which describes the basic principles and terms used in cell culture. The examples in the specification describe the preparation of primary cultures (Example 5) and secondary cultures (Example 6). Thus, use of these terms in the claims is proper [*See In re Wright*, 145 USPQ 182, 188 (CCPA 1965):

> "...the amendments to the specification merely render explicit what had been implicitly disclosed originally, and, while new *language* has certainly been added, we are not prone to view all new "language" ipso facto as "new matter."]

It is believed that the amendments to the claims adequately address the Examiner's rejection with respect to the use of the term "progeny" and "passaging". Accordingly, it is believed that the rejection under § 112, 2nd ¶ is overcome.

Rejection of Claims 17, 18 and 20 under § 112, 1st ¶:

The Examiner's grounds for the rejection of these claims is that the claims are not limited to non-adult tissue and that, in the Examiner's opinion, the specification does not enable methods for proliferating stem cells derived from adult neural tissue.

It should first be pointed out that the specification already states and demonstrates that multipotent neural stem cells obtained from adult tissue were proliferated *in vitro* in the presence of a growth factor (e.g. see Figure 4 and caption, and Example 6). Section 707.07(l) of the MPEP states that "the results of the tests and examples should not normally be questioned by the examiner unless there is reasonable basis for questioning the results."

In paper number 13, the Examiner expressed doubt that the cells proliferated from adult neural tissue were undifferentiated cells because "the presence of nestin expression" was not demonstrated (Paper No. 13). It is not necessary to show nestin immunoreactivity to test for the presence of undifferentiated cells (other characteristics are also indicative, including morphology and growth rate). Nonetheless, the Science article stated that the cells of a neurosphere derived from adult neural tissue are nestin+:

> To determine whether cells within the 6- to 8-DIV spheres could continue to proliferate in secondary cultures, spheres were mechanically dissociated and replated as single cells in the wells of 96-well plates (11). In the presence of EGF, single cells proliferated and formed new spheres (Fig. 1, G through J); the majority of cells within these secondary spheres were also immunoreactive for nestin (10).

(Science article, p. 1708, col. 2) Thus, the Science article describes experiments that demonstrated the *in vitro* proliferation of multipotent neural stem cells obtained from adult neural tissue.

Referring to the Science article, the paragraph bridging the second and third columns on page 1707 states that the ***adult*** mice striata (i.e. ***neural tissue***) were enzymatically dissociated and plated ***in a growth factor-containing culture medium*** (EGF). Most of the cells did not survive the culture conditions, however some of the ***cells began proliferating***. Cell division continued until spheres of proliferating cells formed. Virtually all of the cells in the sphere were immunoreactive for nestin. Continuing onto page 1708 of that article, it states that the spheres were not immunoreactive for neurofilament, neuron-specific enolase, and glial fibrillary acidic protein (i.e. the cells were not immunoreactive for various markers of differentiated neuronal and glial cells, and hence ***the proliferating cells were undifferentiated neural cells***). ***Secondary cultures were prepared*** by plating 200 to 250 of these undifferentiated cells in a 35-mm dish ***containing a fresh growth factor-containing culture medium***. New spheres formed (i.e. ***daughter multipotent neural stem cells proliferated***). Thus, the Science article further supports the specification: that the invention provides a method for the continuous passaging and proliferation of neural stem cells to generate large numbers of undifferentiated neural cells. When the proliferating spheres were provided with a poly-L-ornithine substrate, ***cells*** migrated from the spheres and ***differentiated into neuronal and glial cells*** as evidenced by immunocytochemistry.

The highlighted terms above are the features of claim 17 and claim 92 (use of adult neural tissue). Hence, the Science article demonstrates that a single, multipotent neural stem cell obtained from adult neural tissue can, in the presence of a growth factor, proliferate to form a sphere of

undifferentiated cells which can be subcultured to form new spheres of undifferentiated cells which will, in the presence of suitable culture conditions, differentiate into neuronal and glial cells.

Despite this overwhelming support of the claimed invention, the Examiner maintained the rejection of claim 17 in the present office action stating that "the declaration is not commensurate in scope with the teachings of the specification." (p. 5, lines 8). It should first be emphasized that a Rule 132 declaration traversing a rejection must be commensurate in scope with the ***claims*** (see MPEP § 716, ¶ 7.66, Examiner Note 5). Experimental data provided in a declaration need not duplicate experiments provided in the specification, but rather provide support that the invention works as claimed and that the specification enables the claimed invention.

The Examiner believes that because the declaration states that "***substantially*** the same techniques described in the examples of the specification" were used to proliferate the adult neural stem cells, that it is tantamount to stating that "the methods used were not the same as those methods used in the specification." The term "substantially" was used in the normal context of the word, i.e. that any differences between the experiments described in the specification and the experiments described in the Science article were insignificant. The Examiner states that "the use of bFGF in the reference technique would represent a significant difference" and that "the specification fails to disclose the use of bFGF." While the Examples exemplify the use of EGF to induce proliferation; the specification does disclose bFGF as a proliferation-inducing growth factor (see p.23, lines 12-24). In any event, as discussed above on page 10, the experiments described in the Science article were conducted using EGF as the proliferation-inducing growth factor, the same growth factor used in Example 4 of the specification. Applicants have also demonstrated, in the

specification and supporting declarations, that other growth factors (e.g. TGF, bFGF, and amphiregulin) induce multipotent neural stem cell proliferation. Accordingly, the claimed invention as described in the specification is adequately enabled.

It is believed that it would be unreasonable, and thus contrary to the guidelines of the MPEP § 707.07(l), for the Examiner to continue to question/doubt the substantial amount of supporting evidence which has already been provided by the Applicants. The rejection of Claims 17, 18 and 20 under § 112, 1st ¶, on the grounds that the claims are not limited to non-adult tissue, should be withdrawn.

Rejection of Claim 85 under § 112, 1st ¶:

The Examiner has maintained the rejection that claim 85 is not enabled because "Applicants have failed to disclose evidence that amphiregulin would have the claimed results, which is the proliferation of multipotent stem cells in vitro." (Paper No. 13, lines 3-5).

In response to this rejection, ¶ 10 of the Rule 132 declaration of Dr. Reynolds described an experiment in which neural cells cultured in the presence of amphiregulin formed nestin+, clonally-derived neurospheres. This demonstrated the claimed results – *in vitro* proliferation of a single multipotent neural stem cell in the presence of amphiregulin.

The Examiner found this evidence unpersuasive, stating that "there is no evidence presented that the cells proliferated in response to amphiregulin, lacking adequate controls." The attached Rule 132 declaration of Dr. Reynolds states that in the experiment described in ¶ 10 of the previous declaration, no other growth factors besides amphiregulin were present in the culture medium. Control experiments, using the same culture conditions but without added growth factors, do not induce the proliferation of neural stem

multipotent neural stem cells can be passaged multiple times to produce progeny wherein the progeny are ***multipotent neural stem*** cells." This is indicated by the Examiner's further statements:

> In view of the complexities of cell cycle of stem cells and in view of the problems associated with measuring stem cells (see for example Potten), undue experimentation would be required by one of skill to practice the invention as claimed since there is no evidence in the specification that a second round of passage would result in further proliferation of the stem cell. Note that the examiner has defined the word "progeny" as stem cell daughters which are themselves stem cells.

(Office action, p. 11). Hence, applicants address the Examiner's objection to the specification on the assumption that it is based on the Examiner's disbelief that Applicants have enabled a method to continuously proliferate multipotent neural stem cells *in vitro* using passaging steps.

Applicants first note that the last sentence of the above single-spaced quote is partly correct. As discussed above in connection with the rejections under § 112, 2nd ¶, stem cell progeny includes daughter stem cells. It also includes committed progenitor cells. This is depicted in attached Appendix A as well as in the diagram on page 912 of Exhibit A which was provided with the Preliminary Amendment. Also, page 911 of previous Exhibit A notes that the third defining property of a stem cell is that "when it divides, each daughter has a choice: it can either remain a stem cell, or it can embark on a course leading irreversibly to terminal differentiation (Figure 16-21)."

Contrary to the Examiner's assertion, it would not require undue experimentation for one of skill to practice the invention as claimed. The examples provide detailed directions on how to culture multipotent neural stem cells, and how the cells can be perpetuated. There is ample evidence in the specification that the proliferation of neural stem cell progeny "can be reinitiated at any time... by dissociation of the cells and resuspension in fresh

medium containing growth factors." (p. 26, lines 2-3). The specification repeatedly states that the methods of the invention result in the "perpetuation" of the neural stem cells and the ability to produce "unlimited numbers" of these cells. (see p. 1, line 7; p. 12, lines 15-18; p. 13, line 20; p. 18, lines 3-11; p. 19, lines 5-16; original claim 83; Abstract lines 3-4). Example 6 of the specification also describes the preparation of secondary cultures and the reinitiation of multipotent neural stem cell proliferation, as evidenced by the formation of neurospheres:

> After 6-7 days in vitro, individual cells in the neurospheres from Example 5 were separated by triturating the neurospheres with a fire polished pasteur pipette. Single cells from the dissociated neurospheres were suspended in tissue culture flasks in DMEM/F-12/10% hormone mix together with 20 ng/ml of EGF. A percentage of dissociated cells began to proliferate and formed new neurospheres largely composed of undifferentiated cells.

The statements made in the specification have been further supported by declaratory evidence. In Dr. Reynolds' previous Rule 1.132 declaration, the statement was made that "the cells cultured using the methods described in the application exhibit the characteristics of stem cells..." (Please refer to the single-spaced quote from Dr. Reynolds' declaration which was reproduced above on page 6 under the heading "Summary of the Invention").

The Science article provides even further evidence that the methods of the claimed invention result in the continuous proliferation of multipotent neural stem cells. For the Examiner's convenience, the following text is copied from pages 1708-1709 of the Science article:

> To determine whether cells within the 6- to 8-DIV spheres could continue to proliferate in secondary cultures, spheres were mechanically dissociated and replated as single cells in the wells of 96-well plates (11). In the presence of EGF, single cells proliferated and formed new spheres (Fig. 1, G through J); the majority of the cells within these secondary

continue to question/doubt the substantial amount of supporting evidence which has already been provided by the Applicants. The objection to the specification under § 112, 1st ¶, and the corresponding rejection of Claims 17, 18, 20 and 85-95, on the grounds that there is no support that the claimed methods result in the proliferation of multipotent neural stem cells,

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> spheres were also immunoreactive for nestin (10). When 200 to 250 of these cells were plated in a 35-mm dish, in the presence of EGF and in the absence of supplementary substrate or adhesion factors, 67 ± 4% (n = 3 independent culture preparations) of the cells formed new spheres. As above, if EGF was omitted from the serum-free culture medium, proliferation was not observed. In addition, when EGF was removed from the medium after proliferation had been initiated, no further proliferation was observed. These findings suggest that in vitro conditions may be established for the continual proliferation of undifferentiated cells originally derived from the adult mammalian CNS.... We next examined whether, given a suitable substrate, cells generated from EGF-induced spheres would develop the morphological and antigenic properties of the principal cell types of the CNS. Single 6- to 8-DIV spheres were transferred with micropipettes to poly-L-ornithine-coated glass cover slips... After 21 DIV, the proliferating sphere and cells that had migrated from the core were processed for dual-antigen, indirect immunocytochemistry; both GFAP- (Fig. 2F) and NSE- (Fig. 2G) immunoreactive cells were present. These findings were reproduced in eight independent culture preparations.... Our results demonstrate that EGF induces the proliferation of a small number of cells, isolated from the striatum of the adult mouse brain, that produce clusters of cells with antigenic properties of neuroepithelial stem cells. Under appropriate conditions these cells can be induced to differentiate into astrocytes and neurons with phenotypes characteristic of the adult striatum in vivo.... The ability to induce EGF-responsive stem cells to proliferate in suspension in vitro, and to reinitiate proliferation in a large percentage of the progeny, can provide a plentiful source of undifferentiated CNS cells from the adult...

In summary, the specification adequately describes, teaches, and enables the claimed invention. This has been further supported by supplemental evidence, including the Rule 132 declaration of Dr. Reynolds, which was submitted with the Preliminary Amendment, and the accompanying Science article. Again, it would be unreasonable, and thus contrary to the guidelines of the MPEP [§ 707.07(l)], for the Examiner to

sufficient quantities of certain known growth factors induce the *in vitro* proliferation of multipotent neural stem cells. The neural stem cells do not proliferate in the absence of the added growth factor. The inclusion of this step clearly differentiates Applicants' claimed method from the method described in the Temple paper.

Temple teaches:

> The presence of live conditioning cells is ***critical*** for blast-cell division: when single septal cells are plated into wells containing conditioned medium transferred from striatal cell cultures rather than live conditioning cells, only 1-2% of the septal cells divide, and none of these divide more than once... These results suggest that the conditioning cells are releasing a short-lived, soluble factor important for blast-cell division.

(p. 471, Emphasis added) Applicants' claimed method differs from the Temple method in that, instead of Temple's live conditioning cells, Applicants' culture medium contains "at least one predetermined growth factor capable of inducing multipotent neural stem cell proliferation." Thus, the Temple paper does not anticipate claim 17, as Temple's live conditioning cells secrete unknown amounts of an unknown "soluble factor"; no predetermined growth factors are added to the Temple culture medium.

Furthermore, the Temple paper would not have rendered Applicants' claimed method obvious. This is because the paper suggests that "a short-lived, soluble factor" is required for cell division. Temple teaches away from Applicants' method by showing that by merely adding the soluble factor (using conditioned medium without the live cells) only 1-2% of the neural cells divided only once, and then ceased dividing. Temple could only get multiple rounds of cell divisions by co-culturing the neural cells with the live conditioning cells which apparently continually released the unidentified soluble factor. Thus, Temple teaches away from Applicants' claimed

Claim 95 has been added to further distinguish the claimed method from the Anchan *et al.* method, by reciting that the multipotent neural stem cells proliferated are capable of differentiating into neurons and glia, including astrocytes. Using the method of Anchan *et al.*, only Müller cells were observed (Müller cells are retinal-specific, non-neuronal cells). The presence of astrocytes was not reported.

It appears that, for the § 103 rejection of claim 17, the Examiner used Anchan *et al.* as a primary reference, and relied on Boss to disclose the feature of a passaging step. However, for completeness, Applicants point out that Boss also fails to disclose a method of proliferating multipotent neural stem cells. As with Anchan *et al.*, Boss does not teach or suggest that a single cell can be cultured that is capable of proliferating and producing progeny which can differentiate into neurons and glia. Boss discloses a primary culture of ventral mesencephalon tissue. Approximately 3000 cells are initially plated into each tissue culture well (see col. 9, line 10). It would be expected that each well would initially contain many committed progenitor cells for both neurons and glial cells. Thus, Boss's observations regarding the presence of neurons and glia (quoted by the Examiner in the sentence bridging pages 12 and 13 of the office action) does not show that the Boss culture method results in multipotent neural stem cell proliferation, as these results can be explained by the differentiation of committed neuronal and glial progenitor cells.

The Boss method is clearly designed to select for neuronal progenitor cells (i.e. a cell type that is distinct from multipotent neural stem cells) as the first medium used specifically promotes the survival of neuronal progenitor cells (column 7, last paragraph) and there is no evidence that it promotes survival and/or proliferation of other cell types such as multipotent neural stem cells.

Accordingly, neither Anchan *et al.* or Boss *et al.* teach a key element of Claim 17, the proliferation of a multipotent neural stem cell. Thus, the references, taken together, cannot suggest a method for the proliferation of multipotent neural stem cells, as required by claim 17, and the claims dependent thereon.

With regard to claims 91 and 93, the Examiner apparently views the aggregates described in the Boss patent as being the same as a clonally-derived neurosphere. Using the method of claim 91, a single cell proliferates to form a neurosphere. Because Boss did not culture a single cell, there is no evidence that the aggregates described in col. 7, lines 40-54, formed as the clonal expansion of a single cell. Instead, from this description, it appears as if the aggregates formed from the cells clumping together, as aggregate size was dependent upon initial seeding concentration; the "use of smaller initial seeding concentrations produced small aggregates" (col. 7, line 50). Additionally, col. 11, line 55 states that the cells "clump rapidly upon standing."

With regards to claim 92, the Examiner states that "Boss discloses use of juvenile tissue." For the sake of argument, even assuming that multipotent neural stem cells present in the embryonic or postnatal tissue used in the culture method of Anchan *et al.* proliferated (although, Applicants maintain that they did not), it would not have been obvious that the same results could be duplicated by merely replacing Anchan's embryonic or postnatal tissue with Boss's juvenile tissue. This is because, at the time of the invention, it was not believed that neural stem cells were present in juvenile or adult tissue. With the Preliminary Amendment that was previously filed, Applicants attached as Exhibit E, an article from The New York Times, (March 27, 1992), which demonstrated that researchers in the field of neurobiology were of the belief, at the time of the invention, that

neural stem cells were not found in adult mammalian CNS. The comments made by various researchers in the field of neurobiology, which were quoted in this article, provide compelling secondary evidence of the unobvious nature of the claimed invention. However, in the present office action, the Examiner does not acknowledge that this evidence was even considered. A proper obviousness analysis requires a review of any objective evidence of non-obviousness, including evidence that the results obtained were unexpected. [See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 90 (CAFC 1986), Objective evidence such as commercial success, failure of others, long-felt need and unexpected results must be considered before a conclusion on obviousness is reached ..." (Emphasis added)].

In summary, neither Anchan or Boss demonstrated the proliferation of a single multipotent neural stem cell. Both references described primary cultures, which may or may not have had multipotent neural stem cells present. In any event, even if multipotent neural stem cells were present in the primary cultures of Anchan and Boss, there is no indication that such cells proliferated. Thus a key feature of the claimed invention is missing from both of these references. Accordingly, the combination of Anchan with Boss would not have rendered the claimed invention obvious.

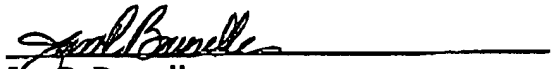
CONCLUSIONS

For the foregoing reasons, it is believed that the specification adequately describes and enables the claimed invention and that, at the time the invention was made, it was unobvious in view of the prior art.

The Examiner is encouraged to telephone the undersigned prior to issuing a further office action to discuss any questions she may have concerning this amendment or to discuss what further actions, if any, are needed to facilitate allowance of the claims.

Respectfully submitted,

FLEHR, HOHBACH, TEST,
ALBRITTON & HERBERT



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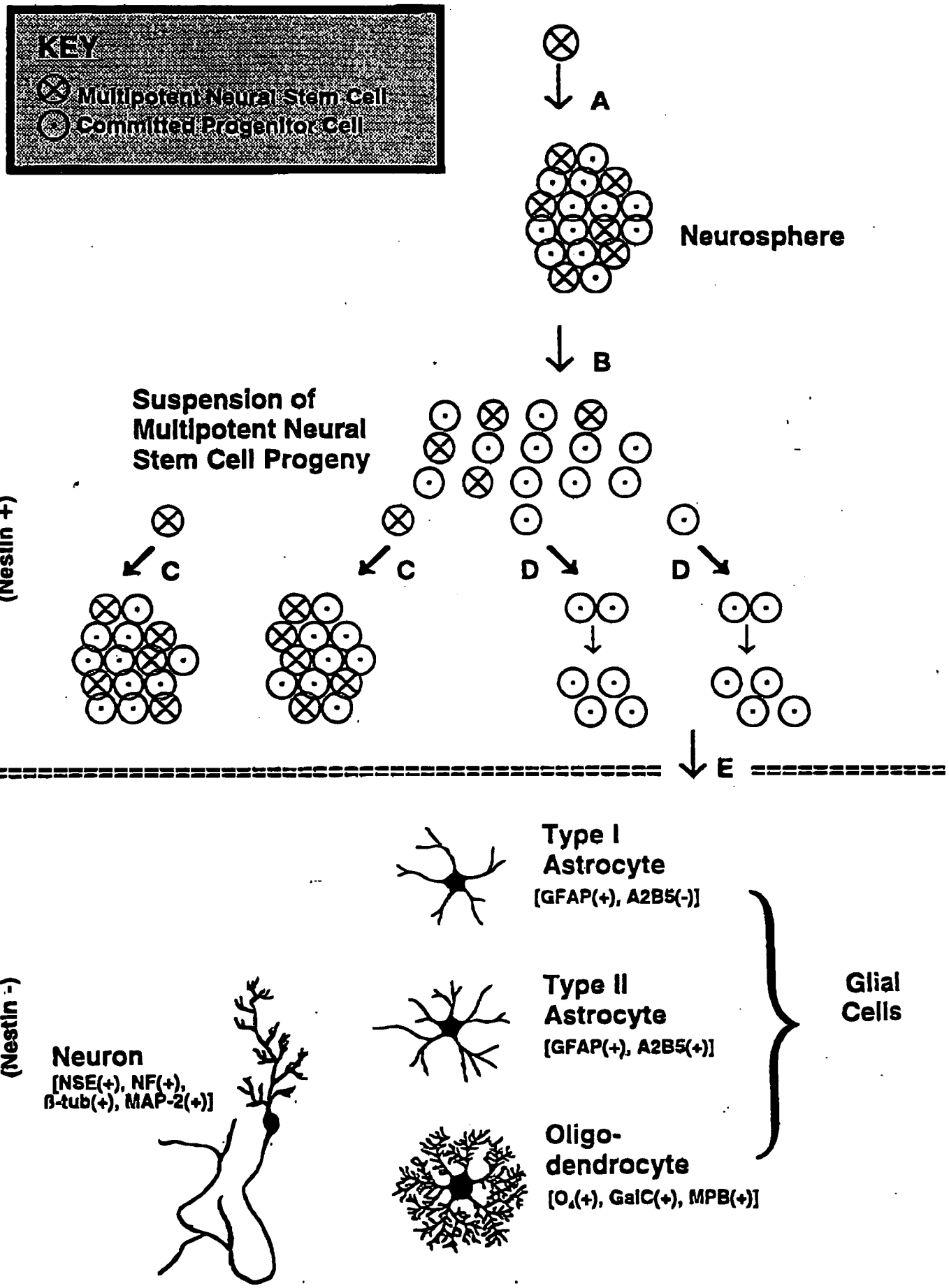
APPENDIX A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Neurosphere
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E
DIFFERENTIATED NEURAL CELLS (Nestin -)
Type I Astrocyte
[GFAP(+), A2B5(-)]
Type II Astrocyte
[GFAP(+), A2B5(+)]
Oligo-dendrocyte
[O4(+), GalC(+), MPB(+)]
Glial Cells
Neuron
[NSE(+), NF(+), ß-tub(+), MAP-2(+)]